Southwest Fisheries Center Administrative Report H-87-19

# HAWAIIAN MONK SEAL DIE-OFF RESPONSE PLAN, A WORKSHOP REPORT
2 April 1980
San Diego, California

William G. Gilmartin
Southwest Fisheries Center Honolulu Laboratory
National Marine Fisheries Service, NOAA
Honolulu, Hawaii 96822-2396

November 1987

## INTRODUCTION

In spring 1978, excessive mortality was observed in the Hawaiian monk seal, Monachus schauinslandi, population at Laysan Island by Johnson and Johnson (1981). While the deaths were occurring, the problem was reported to R. L. DeLong at the National Marine Mammal Laboratory, National Marine Fisheries Service (NMFS), Seattle, Washington. DeLong then put much effort into obtaining an emergency modification of an existing NMFS monk seal permit, organizing a team of scientists to investigate the problem, chartering a vessel to transport the response team, and identifying funds for the die-off investigation. Time, of course, was a critical factor, and although these arrangements were made as expeditiously as possible, the investigation team arrived too late to obtain a good collection of specimens from dying seals and without sufficient latitude in the permit to collect specimens the team believed necessary to resolve the cause of the problem.

The endangered status of the Hawaiian monk seal warrants a thorough investigation into any mortality in the species, especially epidemics similar to this 1978 incident (Gilmartin et al. 1980). A workshop, attended by the members of the 1978 Laysan Island die-off response team (Appendix A) and sponsored by the Marine Mammal Commission, was held on 2 April 1980 in San Diego to develop a contingency plan for response to any future mass mortality in the monk seal population as well as to discuss the 1978 findings and problems (Appendix B). The purpose of this plan is to guide NMFS in application for a permit for thorough monk seal mortality studies in the event of another die-off. This plan and the Marine Mammal Protection Act/ Endangered Species Act (MMPA/ESA) permit should facilitate a prompt and organized response.

### Review of 1978 Laysan Island Die-Off Findings and Problems

A manuscript delineating the findings of the 1978 monk seal die-off (Gilmartin et al. 1980) was delivered at the Symposium on the Status of Resource Investigation in the Northwestern Hawaiian Islands, 24-25 April 1980, University of Hawaii, Honolulu (Appendix C). As discussed by Gilmartin et al. (1980) and Johnson and Johnson (1981), the die-off at Laysan Island probably accounted for at least 50 deaths in the population. Only young (1-5 yr) and old (18-30 yr) animals appeared to have been affected. Investigation into the cause of the mortality included sampling of healthy, sick, and dead individuals. Analyses comprised gross and microscopic pathology, hematology, serum chemistry, virology, bacteriology, parasitology, and toxicology.

All of the dead seals had become emaciated before death, and gross and microscopic pathology indicated that all of the dead seals had gastric ulcerations, of which some were hemorrhaging, associated with heavy gastric nematode infestations. Abnormal histopathology in most other tissues was probably related to the state of malnutrition. No other common pathologic processes were evident, indicating the phenomenon was probably not viral or bacterial in origin, even though two of the live animals tested had significantly elevated total white blood cell counts. Of those two, one

disappeared within 2 wk of sampling, and the other was apparently healthy throughout the summer and during the 1979 season (Johnson and Johnson[1]).

Analyses of liver tissues from the only two freshly dead animals encountered showed high levels of ciguatoxin and maitotoxin, neurotoxins produced by a benthic dinoflagellate in association with tropical reef environments. These toxins affect humans and other mammals that consume certain types of fish in the tropical Pacific. However, the disease signs observed in the dying monk seals were not inconsistent with debilitation by either parasites or ciguatera.

As discussed by Gilmartin et al. (1980), it is not possible at this time to resolve whether the 1978 die-off was due to parasites or ciguatera because "control" animals were not collected for thorough parasite examination or ciguatoxin tissue residue determinations. Not knowing the extent to which apparently healthy monk seals were affected by either parasites or ciguatera at Laysan Island in 1978 led to no defensible conclusions as to the cause of the die-off.

## Ciguatera Study in Elephant Seals

The inability to resolve these questions caused two workshop participants (DeLong and Gilmartin) to propose experimental work with ciguatoxin to determine whether it would affect a phocid and, if so, how. The information collected showed that the northern elephant seal, Mirounga angustirostrus, is extremely sensitive to the toxin (Gilmartin and DeLong 1979).

Eels, Gymnothorax spp., collected in the Northwestern Hawaiian Islands (NWHI) were force-fed, by stomach tube, to two young seals. One seal was acutely poisoned and died within 4 h after a feeding of eel at 6.1% of the seal's body weight. A second elephant seal was more chronically affected and died with a total eel quantity of only 1.7% of its body weight fed over a period of 4 d. This animal began to vomit and experience muscle tremors on the fourth day, refused to consume any more of its normal fish food (Decapterus macarellus), and became weak and unresponsive for 4 d before it died. An electrocardiogram, taken the day before it died, showed heart blocks, which have been observed in humans suffering with ciguatera syndrome.

Workshop participants suggested that research should be performed to provide a better understanding of the impact a heavy parasite load and its associated pathology may have on a phocid. This host-parasite relationship is of importance to much more than resolving the monk seal mortality question because it is a common finding in many pinniped species. It was suggested that the Marine Mammal Commission consider supporting such a study.

---

[1]B. W. Johnson and P. A. Johnson, 8330 SW Alden, Portland, OR 97223, pers. commun., summer, 1979.

## DIE-OFF RESPONSE PLAN

### Initiation of Response

The Marine Mammals and Endangered Species Program of the NMFS, Southwest Fisheries Center (SWFC) Honolulu Laboratory, collects, analyzes, interprets, and reports data on all aspects of Hawaiian monk seal biology. All observed monk seal deaths are reported to the program, and its staff, based on knowledge of the usual conditions and frequency of reported mortality, should make the determination concerning mobilization of a response team to investigate unusual mortality and, as possible, treat affected seals.

### Response Team Members

The team that will respond to investigate a die-off, as determined above, should contain as a minimum the following:

1. A biologist, knowledgeable of normal Hawaiian monk seal behavior, age classification, and beach use patterns in the NWHI.

2. A veterinarian familiar with pinniped diseases, or if such expertise is not available for travel, arrangements must be made for such a person to be in radio communication with the response team.

3. A veterinary technician or a person knowledgeable in techniques of collection of specimens for pathology, toxicology, and microbiological studies and capable of performing certain clinical laboratory procedures such as blood counts.

4. A marine mammal parasitologist or a person trained in collection, fixation, and identification of common pinniped parasites, because of the unusually high parasite load observed in monk seals examined to date and the potential that parasites may at least be a complicating factor in any observed mortality.

5. The team should total at least five individuals because large seals may require restraining and sampling. Ideally, all members should have at least some expertise in monk seal biology or veterinary pathology to enable collection of as much information as possible in the field that may be related to ongoing mortality.

### Equipment Requirements

Equipment and supply requirements (not including those necessary for basic subsistence and transportation) for a field team to fully investigate and collect specimen materials are presented in Appendix D. Quantities of some of these materials will vary with the number of animals to be examined,

and this should be taken into account in preparation of a field kit. The kit outlined in Appendix D was designed to live sample or necropsy up to 25 monk seals.

Other collection kits also should be prepared for use by field personnel for collection of specimen material from any dead animals that may be found in the course of their routine field research but are not part of a die-off. The value of these opportunistic samplings will vary greatly with the state of decomposition of any individual but should, in the long term, develop some basic information on clinical pathology and age-related reproductive characteristics.

## Logistics and Funding Support

Both the logistical support and funding requirement for a monk seal die-off response will vary greatly by location of the die-off. Military flights from Honolulu are presently available weekly to Midway Island and bimonthly to Kure Atoll. Charter flights can be arranged to Tern Island at French Frigate Shoals. Access to military flights and arrangements for a charter aircraft flight can be accomplished easily within a few days. The NOAA ship Townsend Cromwell is assigned to the SWFC and may be used to support a monk seal die-off investigation. Use of the Townsend Cromwell would, if it was working in the Hawaiian Archipelago, require approval of the Director of the NMFS, SWFC, Honolulu Laboratory, and the Pacific Marine Center approving officials. The most expensive travel would occur if it became necessary to charter a commercial vessel to transport the response team and associated equipment. Should a charter vessel be required, the contract would be awarded with a minimum delay by using the procedure outlined in Appendix E. Limited funding, as required, from outside of the SWFC operating monk seal research budget, will be provided by other NMFS funding resources (Appendix F).

## Permit Requirements

The MMPA/ESA permit, which will allow an investigation as outlined in this plan, should enable collection of specimens and animals as described below:

1. Necropsy and collect tissues and skeletal materials from all dead animals.

2. Take moribund animals by sacrificing, in a humane manner, only if sufficient freshly dead seals are unavailable for autopsy or additional information will aid in diagnosis. Only seals identified by clinical signs to be suffering the same disease phenomena as those that have died will be taken. The number of seals taken in this manner will be based on consistency of the pathology and diagnostic value of the specimens but will not exceed five animals in any one disease investigation.

3. Up to 10 seals with signs of the disease being investigated will be marked with bleach and flipper tagged (if not previously tagged) to permit the animals to be reliably monitored for possible recovery and to document the development of the disease characteristics. These animals also may be physically restrained for blood and culture collection and body temperature testing up to three times each.

4. Physically restrain and collect blood, culture swabs, stool, and body temperature by rectal probe from up to five apparently healthy animals of the same age/sex class as that of affected animals, at each island where the disease is apparent.

5. Restrain and collect liver and blubber biopsies from half of the animals in item 4--only if it is determined that the cause of the die-off being investigated may be a toxic substance and control tissue levels would be essential to a diagnosis.

6. Take, by sacrificing in a humane manner, up to three apparently healthy males of the affected age class--only if it is determined by the veterinary pathologist that thorough examinations of these control seals are critical to making a diagnosis, and following consultation with the entire response team, a majority decision to take these animals is reached. This collection would be considered only if specimens in items 4 and 5 above are insufficient as controls.

7. When the cause of the mortality is apparent or when specific treatments may aid in diagnosis of the problem and appropriate veterinary medical supplies are available, experimentally treat some affected animals, while maintaining an untreated control group. If the treatment is effective, treat additional monk seals if signs developing in other individuals indicate the same disease process is affecting them.

## Field Procedures and Laboratory Studies

Weeks may easily ensue between time of field inspection of a problem and obtaining complete laboratory results on submitted specimens. This is, of course, long enough for mortality to have subsided or to have significantly expanded. With either course, it is critical that as much information and specimen material as possible be collected on any single field trip to investigate mass mortality.

When the disease investigation team arrives at the site of reported mortality, it should begin with a thorough documentation of the signs displayed by affected animals; their chronology, including duration of time from apparent onset of signs to death; and age classes affected. A necropsy should be performed on all freshly dead animals. A necropsy procedure, suitable for use on the Hawaiian monk seal, was prepared by Griner for use in these mortality studies (Appendix G). Tissues should be

collected in formalin, parasites that may be found should be placed in alcohol or a parasite fixative, and abnormalities if found should be noted. A list of specimens to be collected at the necropsy of a monk seal, the method of preservation for transport to the laboratory, and the tests that will be performed on them are contained in Appendix H. Cause of the mortality may seem apparent following necropsy of several animals. In such situations, the entire set of specimens listed should still be collected from as many seals as possible to confirm the diagnosis and ensure that no other pathological conditions were overlooked.

If information from these dead seals is insufficient to make a diagnosis or dead specimens of good quality are not available, moribund seals should be sacrificed in a humane manner for necropsy. Following these necropsies, if the cause of mortality is still uncertain and the team veterinarian (in attendance or by radio consultation) determines that examination and blood and culture sampling of healthy seals of the affected age classes may aid in diagnosis, then these animals should be sampled as outlined in the MMPA/ESA permit. Liver and blubber biopsies also may be required if some type of toxic agent is suspected.

If the cause is still unclear and it is the professional opinion of the team veterinarian that control seals must be collected to differentiate between endemic or subclinical diseases in the monk seal population and that which is causing the mass mortality of concern, and the majority of the response team agrees, then a limited number of male seals from the affected age classes may be taken for necropsy.

When the cause of the mortality is apparent or when specific treatments may aid in diagnosis of the problem and appropriate veterinary medical supplies are available, some affected animals should be experimentally treated while maintaining an untreated control group. If treatment is effective, treat additional monk seals if signs developing in individuals indicate the same disease process is affecting them.

## Author's Note

This plan was used by the SWFC to obtain MMPA/ESA Permit 413, which fully incorporates the sampling priorities and protocols that the April 1980 workshop participants recommended. In addition, Permit 413 allows collection of sick seals for observation, treatment, and rehabilitation at an approved captive seal facility.

## LITERATURE CITED

Gilmartin, W. G., and R. L. DeLong.
1979. Investigation of unusual mortality in the Hawaiian monk seal, Monachus schauinslandi. Abstracts from Presentations at the Third Biennial Conference of the Biology of Marine Mammals, October 7-11, 1979, Seattle, Washington, p. 24.

Gilmartin, W. G., R. L. DeLong, A. W. Smith, L. A. Griner, and M. D. Dailey.
1980. An investigation into unusual mortality in the Hawaiian monk seal, Monachus schauinslandi. In R. W. Grigg, and R. T. Pfund (editors), Proceedings of the Symposium on Status of Resource Investigations in the Northwestern Hawaiian Islands, April 24-25, 1980, University of Hawaii, Honolulu, p. 32-41. UNIHI-SEAGRANT-MR-80-04.

Johnson, B. W., and P. A. Johnson.
1981. The Hawaiian monk seal on Laysan Island: 1978. U.S. Dep. Commer., Natl. Tech. Inf. Serv., PB82-109661, 17 p.

Appendix A

**PARTICIPANTS, WORKSHOP TO DEVELOP A HAWAIIAN MONK SEAL DIE-OFF RESPONSE PLAN**

Dr. Murray D. Dailey
Department of Biology
Long Beach State University
Long Beach, California 90801

Robert L. DeLong
National Marine Fisheries Service
National Marine Mammal Laboratory
7600 Sand Point Way, N.E.
BIN C15700
Seattle, Washington 98115

William G. Gilmartin (chairperson)
National Marine Fisheries Service
Southwest Fisheries Center Honolulu Laboratory
2570 Dole Street
Honolulu, Hawaii 96822-2396

Dr. Lynn A. Griner
Zoological Society of San Diego
P.O. Box 551
San Diego, California 92113

Dr. Alvin W. Smith
School of Veterinary Medicine
Oregon State University
Corvallis, Oregon 97331

Appendix B

**AGENDA, WORKSHOP TO DEVELOP A HAWAIIAN MONK SEAL DIE-OFF RESPONSE PLAN**

1. Introduction

   a. Statement of objectives (Gilmartin)

   b. Review of 1978 Laysan die-off finding problems

   c. Status of ciguatera study in elephant seals

2. Development of Die-Off Response Plan

   a. Guidelines for identification of unusual mortality

   b. Discussion of specimens and field data to be collected and the rationale for them

   c. Consideration of specimen and data collection from "control" seals

   d. List of equipment, supplies, necropsy kits

   e. Discussion of permit requirements

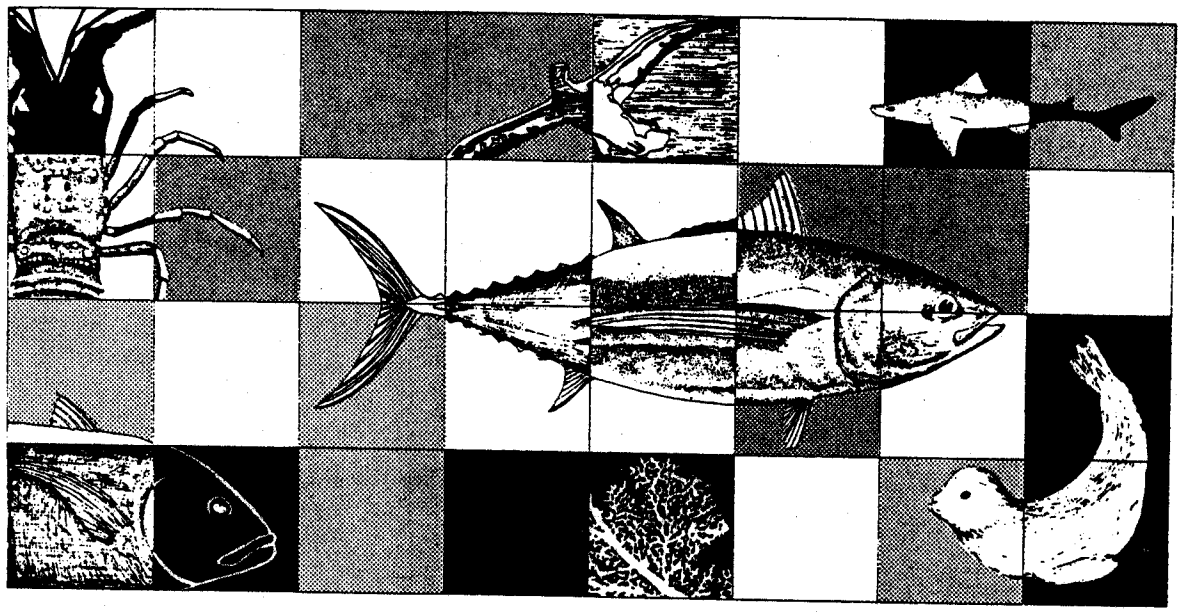
   f. List of required on-site personnel

   g. Logistics support

   h. Estimate costs of response, to include transportation of personnel and supplies, salaries, consultation fees, equipment and supplies, and any other costs might be incurred.

Reprinted From the Proceedings of the Symposium on

# STATUS OF RESOURCE INVESTIGATIONS IN THE NORTHWESTERN HAWAIIAN ISLANDS



(UNIHI-SEAGRANT-MR-80-04),
April 24-25, 1980 Campus Center Ballroom,
University of Hawaii, Honolulu, Hawaii

Editors

Richard W. Grigg

Rose T. Pfund

DATE: August 1980

PUBLISHED BY: University of Hawaii Sea Grant College Program
Honolulu, Hawaii 96822

SPONSORED BY: National Marine Fisheries Service Honolulu Laboratory
Hawaii Division of Fish and Game
University of Hawaii Sea Grant College Program
U.S. Fish and Wildlife Service Pacific Islands Area Office

# AN INVESTIGATION INTO UNUSUAL MORTALITY IN THE HAWAIIAN MONK SEAL, MONACHUS SCHAUINSLANDI

William G. Gilmartin, Robert L. DeLong, Alvin W. Smith, Lynn A. Griner, and Murray D. Dailey

Southwest Fisheries Center Honolulu Laboratory, National Marine Fisheries Service, NOAA, P.O. Box 3830, Honolulu, Hawaii 96812; National Marine Mammal Laboratory, National Marine Fisheries Service, NOAA, Seattle, Washington 98115; Naval Ocean Systems Center, San Diego, California 92152; Zoological Society of San Diego, San Diego, California 92112; Southern California Ocean Studies Consortium, Long Beach, California 90801

## ABSTRACT

Increased mortality was reported in the endangered Hawaiian monk seal population at Laysan Island in the spring of 1978. An investigation of the possible causes of the mortality included sampling of healthy, sick, and dead individuals. Analyses comprised gross and microscopic pathology, hematology, serum chemistry, virology, bacteriology, parasitology, and toxicology. Gastric ulceration in varying degrees due to nematodes was a consistent finding. Evidence of caliciviruses (VESV and SMSV) and Salmonella was found in the population. Two of 18 seals tested had elevated total white blood cell counts. A few individuals differed significantly from mean serum chemistry values but no trend was apparent. Liver tissues of two seals tested for ciguatoxin and maitotoxin were positive.

Monachus schauinslandi
mortality
clinical pathology
ciguatera

## INTRODUCTION

The Hawaiian monk seal, Monachus schauinslandi, is an endangered species which breeds only in the Northwestern Hawaiian Islands from

Necker Island west to Kure Atoll. Recent censuses indicate the total population has decreased by about 50% since 1958 (Johnson et al., in preparation).

In the spring of 1978, high mortality was observed in monk seals at Laysan Island (B.W. Johnson and P.A. Johnson, Aquatic Mammals Behavioral Research Company, Honolulu, Hawaii 96822, personal communication, 1978). Disease signs apparent in the monk seals were consistent. Animals come ashore emaciated or began to noticeably lose weight as they lay on the beach. The seals abandoned normal hauling out behavior--failing to move into the vegetation behind the beach crest at night. Within 2 to 3 weeks of beginning the weight loss, the animals became completely debilitated and then died in the splash zone or at the high tide line (B.W. Johnson and P.A. Johnson, personal communication, 1978).

This report discusses data collected on specimens taken from 19 dead and 18 live monk seals during April and May 1978 as part of an investigation into the reported mortality.

## MATERIALS AND METHODS

Between 4 May and 13 May 1978 we collected specimens and data from a total of 24 Hawaiian monk seals at two locations in the Northwestern Hawaiian Islands to determine if there was any apparent disease process in the seals which might cause the mortality. Ten live yearlings or juvenile seals (MS-01-78 to MS-10-78) and one adult (MS-11-78) were sampled at Laysan Island. In addition, we received tissue sets in formalin[1] from 13 monk seals (collected by Brian W. and Patricia A. Johnson on Laysan Island, 1 March to 1 May 1978) which died at Laysan Island prior to our arrival. Samples were to be collected from a large number of sick as well as apparently healthy animals; however, a storm just prior to our arrival cleared the beaches at Laysan Island of most of the very sick animals with the disease signs mentioned earlier. Seal MS-11-78 was very emaciated and weak and died while being restrained for collection of the samples. At French Frigate Shoals six dead seals were found, only one of which was fresh enough to be necropsied, even through it had been dead at least a day and the tissues were badly autolysed. The other five were too decomposed to yield any information relative to cause of death.

The live animals were physically restrained and blood was collected from the intra-vertebral extradural vein. Packed red cell volumes and white blood cell counts were determined in the field. Serum and plasma for the other clinical bood tests and serological studies were frozen for later analysis. Clinical chemistry tests were performed using standard laboratory procedures (Bio-Science Laboratory, Van Nuys, California). Serum samples from all animals were tested for agglutinating

[1]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

antibodies to Leptospira antigen pools nos. 1, 2, and 3.[2] They were also tested for serum neutralizing (SN) antibodies to 19 calicivirus types (vesicular exanthema of swine virus types $A_{48}$, $C_{52}$, $D_{53}$, $F_{54}$, $G_{55}$, $I_{55}$, $J_{56}$, $K_{56}$, San Miguel sea lion virus types 1, 2, 4, 5, and marine calicivirus isolates designated 427, 274, fluke, V86, 804T, and 913T) using previously described microtiter techniques (Monta and Bryon, 1974; Smith et al., 1976).

Leptospira isolation attempts were made on samples of liver, kidney, and cerebrospinal fluid taken from MS-11-78. The procedure has been previously described (Smith et al., 1974a; Smith et al., 1974b).

Salmonella incidence in the seals was tested by collection of rectal swabs from all animals and placing them into transport medium in the field. These cultures were tested by one of the authors (Gilmartin) for salmonellae by beginning enrichment and isolation procedures previously described (Gilmartin et al., 1979) within 24 hours of collection of the sample but were also maintained in the holding medium for over 2 weeks, when another attempt was made at isolation of salmonellae (N.A. Vedros, Naval Biosciences Laboratory, Oakland, California 94625, personal communication, 1978).

Swabbings were taken from the nose, throat, and rectum of each animal for virus isolation. These and small slips of lung, liver, kidney, and tonsil from animal no. 11 were placed in ampules of phosphate-buffered glycerine, pH 7.2, and immediately frozen to -55°C. Tissues were thawed and ground up, then they and the swab samples were clarified by centrifugation at 3,000 rpm. Supernatant fluids were placed in a Vero monkey, Cercopithicus aethiops, kidney cells and porcine kidney cells (PK-15), incubated at 37° and 30°C, and passaged at least four times as previously described (Smith et al., 1974b).

Stool specimens were collected, as available, from the seals and frozen for later flotation and examination for ova.

Rectal temperatures were determined using an electronic thermistor with a flexible probe inserted at least 30 cm through the rectum.

Tissues for microscopic histopathologic studies were preserved in formalin and examined after hematoxylin and eosin staining.

One canine tooth was extracted from each of the dead seals for aging using a new technique developed for small cetaceans (Pierce and Kajimura, 1978).

Liver specimens from two seals (MS-11-78 and MS-12-78) were assayed for dioxan (2, 3, 7, 8 - tetrachlorodibenzo-p-dioxan) using gas chromatography and high resolution mass spectrometer techniques (M. Gross, University of Nebraska, Lincoln, Nebraska 68588, personal communication, 1978.)

---

[2]Difco Laboratories, Detroit, Michigan. These pools contain Leptospira ballum, L. canicola, L. icterohemorrhagiae, L. bataviae, L. grippotyphosa, L. pyogenes, L. autumnalis, L. pomona, and L. wolffii.

Tests for tissue residues of ciguatoxin by radioimmunoassay procedures (Hokama et al., 1977) and ciguatoxin and maitotoxin by bioassay techniques were performed.[3]

Statistical analyses were performed on the clinical chemistry, hematology, and temperature data to test for individuals with values different than the mean. All the live seals sampled with one exception were young (yearlings to juveniles); therefore the adult (MS-11-78) was excluded from these statistical tests and the mean and standard deviation of the data from these animals were taken as a close approximation to parametric values since normals for the population were not known. The farthest-outlying variates within a given sample (i.e., sodium) were then tested to see if they statistically belonged within that sample using a one-tailed t-test of one variate against the assumed population mean (Sokal and Rohlf, 1969). Also, because of the great distance between the islands (approximately 320 nmi), the data for the young monk seals from Laysan Island (MS-01-78 through MS-10-78) were tested with a two-tailed Wilcoxon two-sample test (Sokal and Rohlf, 1969) against those from French Frigate Shoals (MS-13-78 through MS-19-78) for all categories to determine if there were differences between the island populations.

## RESULTS AND DISCUSSION

Animals from two age groupings, the young and very old, were represented in the dead animals which were recovered by the Johnsons and the authors. Ten of 14 seals which died and were recovered at Laysan Island were between 1 and 5 years of age; the others were between 18 and 30 years. The net loss in monk seals at Laysan Island during the period from March to July 1978 is estimated to be at least 50 animals (Johnson and Johnson, 1980).

Of the 13 seals which had died prior to our arrival at Laysan Island and from which we received tissue sets, there were seven males and six females. Four of the six dead seals at French Frigate Shoals were females and sex could not be determined for the other two.

Twelve of the live young monk seals sampled were females, five were males and the single adult at Laysan Island (MS-11-78) was a male. Four of the seals sampled at Laysan Island (MS-01, MS-04, MS-07, and MS-10) became emaciated and disappeared by mid-June 1978. The seals sampled at French Frigate Shoals were not similarly monitored.

Statistical tests were performed on the clinical data to identify animals with test results significantly different ($P \leq 0.05$) from the mean of all monk seals sampled. The tests were done to aid in recognizing ill animals in a species for which these clinical parameters had not been determined.

---

[3]Radioimmunoassay for ciguatoxin and bioassay for ciguatoxin and maitotoxin were performed by Dr. Y. Hokama and Dr. J. Miyahara, respectively, at the University of Hawaii, John A. Burns School of Medicine, Pathology Department, Honolulu, Hawaii 96822.

When each individual seal's hematology and clinical chemistry test results were compared to the mean of the group, many had at least one test value different from the mean (Table 1). There were only three cases where two animals differed from the mean ($P \leq 0.05$) in the same direction on the same test: MS-05 and MS-07, elevated total white cell count; MS-06 and MS-18, high cholesterol; and MS-08 and MS-15, high alpha-1 globulin.

Monk seals MS-05 and MS-07, with the high white cell counts, are noteworthy because MS-07 is one of four animals which disappeared and presumably died later in the season. MS-07 had the highest white cell count (18,700) of all seals tested and was one of three young seals sampled which appeared underweight and lethargic. Neither of these two with the elevated total white cell counts had any other outstanding clinical data values. Of the other two monk seals which appeared underweight at sampling, one (MS-06) had only a significantly elevated cholesterol and glucose level, which may indicate a fasting animal, and the other (MS-01) had no clinical blood tests different from the mean of the group.

The three other animals which disappeared in an emaciated condition during the summer, MS-01, MS-04, and MS-10, did not exhibit any remarkable findings except for a high lactic dehydrogenase (LDH) in MS-10.

The only other animals with any noteworthy abnormal clinical pathology were MS-09 with a high total protein and beta globulin and MS-16 with a very low packed red cell volume and a high serum glutamic pyruvic transaminase (SGPT). Salmonella sieburg was isolated from a rectal swab taken from MS-09 and it is the only seal from which salmonellae were recovered (N.A. Vedros, personal communication, 1978). Although Salmonella are common isolates in some pinnipeds (Gilmartin et al., 1979), the high beta globulin and total protein in this animal are probably not related to a chronic infectious bout with this organism as no serum antibody could be detected (N.A. Vedros, personal communication, 1978).

The high SGPT of MS-16 would indicate some liver pathology. The low hematocrit may be due to hemorrhage associated with severe gastric ulceration due to nematode infestation which will be discussed below. Despite the frequency and apparent severity of these parasitic ulcerations observed in dead animals, MS-16 was the only living seal tested which had a low packed cell volume.

The rectal temperature statistics in Table 1 show that all animals tested were within a range of 1.5°C. All of these animals were asleep and dry when initially approached so there had probably been little or no physical activity prior to our restraining them. Thus, these temperatures (with a mean of 36.3°C) reflect resting status, and are very close to that previously reported for young Hawaiian monk seals. Several monk seals were monitored throughout the restraint period, and no change in the temperature reading was noted. Temperatures taken by various means in some other phocids are reported between 36.0°C and 37.0°C.

TABLE 1. SERUM CHEMISTRY, BLOOD COUNTS, AND RECTAL TEMPERATURE STATISTICS FROM 17 YOUNG HAWAIIAN MONK SEALS, 1978

| Test | Mean | Standard Deviation | Range | Animals Significantly Different at $P \leq 0.05$ | |
|---|---|---|---|---|---|
| Sodium (meq/liter) | 152.7 | 8.1 | 134-167 | MS-08 (134) | MS-18 (167) |
| Potassium (meq/liter) | 5.84 | 0.63 | 4.6-7.0 | MS-03 (7.0) | MS-08 (4.6) |
| Chloride (meq/liter) | 108.7 | 4.8 | 96-119 | MS-08 (96) | MS-13 (119) |
| Calcium, total (meq/liter) | 5.54 | 0.37 | 5.0-6.1 | | |
| Inorganic phosphorus (mg/100 ml) | 7.49 | 1.49 | 5.3-9.6 | | |
| Cholesterol (mg/100 ml) | 206.7 | 54.5 | 121-314 | MS-06 (314) | MS-18 (311) |
| Urea nitrogen (mg/100 ml) | 37.1 | 12.3 | 21-63 | MS-17 (63) | |
| Uric acid (mg/100 ml) | 2.74 | 0.51 | 1.7-3.4 | MS-02 (1.7) | |
| Bilirubin, total (mg/100 ml) | 0.38 | 0.28 | 0.2-1.2 | | |
| Alkaline phosphatase (units) | 222.0 | 131.2 | 74-580 | | |
| LDH (units) | 758.9 | 454.7 | 62-1,640 | | |
| SGPT (units) | 137.8 | 57.6 | 76-290 | MS-16 (290) | |
| SGOT (units) | 146.9 | 45.7 | 72-220 | | |
| Glucose (mg/100 ml) | 91.1 | 24.6 | 49-141 | MS-06 (141) | |
| Total protein (g/100 ml) | 7.32 | 1.01 | 4.9-9.5 | MS-09 (9.5) | |
| Albumin (g/100 ml) | 2.82 | 0.36 | 34 | | |
| Alpha-1 globulin (g/100 ml) | 0.32 | 0.33 | 0.08-1.2 | MS-08 (1.2) | MS-15 (0.9) |
| Alpha-2 globulin (g/100 ml) | 1.09 | 0.53 | 0.4-1.96 | | |
| Beta globulin (g/100 ml) | 0.80 | 0.25 | 0.4-1.3 | MS-09 (1.3) | |
| Gamma globulin (g/100 ml) | 2.30 | 0.57 | 1.3-3.4 | MS-06 (3.4) | MS-08 (1.3) |
| Albumin/globulin ratio | 0.64 | 0.09 | 0.5-0.8 | | |
| Packed red cell volume (5) | 57.1 | 4.9 | 46.0-62.5 | MS-16 (46.0) | |
| White cell count, total (cells/$mm^3$) | 9,745 | 3,178 | 5,170-18.700 | MS-05 (15,400) | MS-07 (18,700) |
| Rectal temperature (°C) | 36.3 | 0.54 | 35.5-37.0 | | |

Rectal swab cultures from more than half of the animals yielded Edwardsiella tarda which is of dubious significance as an intestinal tract pathogen.

Neither viruses nor leptospires were isolated from any sample; however, animal MS-05 did carry SN antibodies against VESV $I_{55}$ at the 1:40 dilution and animals MS-13 and MS-19 carried SN titers of 1:10 against SMSV-1. All other tests for virus and Leptospira antibodies were negative; however, the finding of calicivirus antibodies (VESV and SMSV) in 3 of 18 animals certainly suggests occasional contact with these agents and may be some indication that virus reservoirs exist along the Northwestern Hawaiian Islands chain. Alternatively, northern elephant seals, Mirounga augustirostris, have been reported as far west as Midway Islands (M.J. Rauzon, National Fish and Wildlife Laboratory, Anchorage, Alaska 99503, personal communication, 1978), the western limit of the monk seal range, and caliciviruses have been isolated repeatedly from nursing and weaned elephant seals along the southern California coast (A.W. Smith, Naval Biosciences Laboratory, Oakland, California 94625, personal communication, 1978, 1979). Although there is no evidence to suggest that the recent die-off was in any way related to the presence of caliciviruses, it should be remembered that these agents have been associated with a vesicular disease and reproductive failure in California sea lions, Zalophus californianus, northern fur seals, Callorhinus ursinus, and domestic swine and cats.

Parasite ova found in the stool of the 10 young live seals at Laysan Island are described in Table 2. The following flatworm ova were recovered from the gastrointestinal tract of the adult (MS-11-78) which died at Laysan Island: Corynosoma rauschi, Contracaecum turgidum, Diphyllobothrium cameroni, D. elegans, and D. hians. Contracaecum turgidum, Corynosoma rauschi and D. hians were found in the stomach and intestines of MS-12-78 at French Frigate Shoals. These same parasite species were represented in many of the 13 animals which died at Laysan Island between 1 March and 1 May 1978.

All of the animals from which the tissue sets were collected, including the two examined by the authors, were cachetic and severely emaciated. Common findings in these 15 animals included: heart, lack of adipose tissue on the epicardium surface; liver, centralobular congestion, with foci of centralobular necrosis; lungs, congestion and alveolar hemorrhage in about half of the seals; spleen and lymph nodes, little or no evidence of lymphopoietic activity; testes, no evidence of spermatogenesis in males estimated to be subadult to adult; and, gastrointestinal tract, numerous foci of ulceration (many were actively hemorrhaging) with nematodes embedded deep into the stomach wall in all animals and many had additional intestinal lesions from cestodes, similarly embedded in the mucosa.

It is important to note that in December 1978, two additional monk seals were found dead at Laysan Island (B.W. Johnson and P.A. Johnson, personal communication, 1978) in an emaciated condition resembling that seen in April and May; however, these seals, on examination, had very light gastric nematode infestations and only minor ulceration at the phylorus.

TABLE 2. PARASITE OVA IN STOOL OF YOUNG LAYSAN MONK SEALS, 1978

| Monk Seal | Cestode Ova* | Capillorid Type Ova* |
|---|---|---|
| MS-01 | M | -- |
| MS-02 | H | L |
| MS-03 | H | -- |
| MS-04 | M | L |
| MS-05 | N.D. | N.D. |
| MS-06 | N.D. | N.D. |
| MS-07 | N.D. | N.D. |
| MS-08 | H | -- |
| MS-09 | H | -- |
| MS-10 | H | -- |

*Number of ova in 400 power microscope field: L (light) = <25, M (moderate) = 25 to 75, H (heavy) = >75, N.D. = not determined

The extensive pathology caused by parasites, even though common to all of the monk seals which were necropsied during the period of high mortality in the spring, may be the result of seasonal fluctuations in gastric nematode parasite load and not, necessarily, a major factor in the spring 1978 mortality. Gastric nematode infestations, many with associated ulcerations, are relatively common in pinnipeds and since gastrointestinal tracts of only emaciated animals were examined, it is not possible to know the associated parasite pathology in the "normal" population. Table 2, however, indicates many of the apparently normal seals were carrying heavy cestode loads.

No dioxan was detected in the liver samples tested. Ciguatoxin and maitotoxin bioassay analyses of liver tissues from the adult which died at Laysan Island (MS-11-78) and the juvenile at French Frigate Shoals (MS-12-78) were positive. Estimated levels were 30 to 50 times that found in the liver of a control monk seal which had been maintained in captivity for 15 years. Radioimmunoassay for ciguatoxin in the same tissues revealed the liver of MS-11-78 to be about 25% above the control liver, while MS-12-78 was 9% below the control. Subsequent studies, the results of which will be published elsewhere, have shown that eels (known to be a part of the monk seal diet), collected near the islands on which the monk seals haul out, can debilitate and kill northern elephant seals after consumption of as little as 1.7% of the animal's body weight (DeLong and Gilmartin, in preparation).

The parasite associated pathology and the presence of ciguatoxin in the animals were the major findings which might account for this die-off of monk seals. Lack of any pathology in any organ systems (other than gastrointestinal) may discount any infectious disease processes of viral or bacterial origin.

Further study is needed to assess the impact of heavy gastrointestinal parasitism on pinnipeds relative to their general health and ability to feed and otherwise function normally. The signs displayed by the dying monk seals observed at Laysan Island are not inconsistent with what might be expected if the parasites were responsible, but they also could have been caused by the ciguatera syndrome. Ciguatera, which will kill a phocid seal, is known to be present in tropical reef environments and is present in the island chain in at least one of the monk seals' food fish. Continued disease monitoring of the seal population and experimental work in parasitology and ciguatera toxicology will be necessary to resolve the impact of these on the Hawaiian monk seal.

REFERENCES

DeLong, R.L., and W.G. Gilmartin. Ciguatoxin feeding experiments with a model phocid seal. National Marine Mammal Laboratory, Northwest and Alaska Fisheries Center, National Marine Fisheries Service, NOAA, Seattle, Washington 98115. In preparation.

Gilmartin, W.G., P.M. Vainik, and V.M. Neill. 1979. Salmonellae in feral pinnipeds off the southern California coast. Journal of Wildlife Diseases 15(4):511-514.

Hokama, Y., A.H. Banner, and D.B. Boylan. 1977. A radioimmunoassay for the detection of ciguatoxin. Toxicon 15:317-325.

Johnson, A.M., R.L. DeLong, C.H. Fiscus, and K.W. Kenyon. Status of the Hawaiian monk seal population, 1978. National Marine Fish and Wildlife Laboratory, Anchorage, Alaska 99503. In preparation.

Johnson, B.W., and P.A. Johnson. 1980. The Hawaiian monk seal on Laysan Island, 1978. Draft report to the Marine Mammal Commission, Washington, D.C. 20006, 39 pp.

Monta, A.S., and E.R. Bryon. 1974. Micro-neutralization test for detection of rhinovirus antibodies. Proceedings of the Society for Experimental Biology and Medicine 145:690-694.

Pierce, K.V., and H. Kajimura. 1978. Acid etching and highlighting for defining growth layers in cetacean teeth. Proceedings of the International Conference on Determining Age of Odontocete Cetacean. La Jolla, California, 5-7 September 1978.

Smith, A.W., R.J. Brown, D.E. Skilling, and R.L. DeLong. 1974a. Leptospira pomona and reproductive failure in California sea lions. American Journal of Veterinary Medical Association 165:996-998.

Smith, A.W., C.M. Prato, W.G. Gilmartin, R.J. Brown, and M.C. Keyes. 1974b. A preliminary report on potentially pathogenic microbiological agents recently isolated from pinnipeds. Journal of Wildlife Diseases 10(1):54-59.

Smith, A.W., T.G. Akers, C.M. Prato, and H. Bray. 1976. Prevalence and distribution of four serotypes of SMSV serum neutralizing antibodies in wild animal populations. Journal of Wildlife Diseases 12:326-334.

Sokal, R.R., and F. J. Rohlf. 1969. Biometry: The Principles and Practice of Statistics in Biological Research. San Francisco: W.H. Freeman and Company, 776 pp.

Appendix D

## MASS MORTALITY SPECIMEN COLLECTION KIT

| Item | Quantity (minimum) | Remarks |
|---|---|---|
| Gloves, rubber | 4 pairs | Heavy rubber with rough hand surface |
| Knives, 6" blade | 2 | |
| Scissors | 2 | At least one pair of blunt - sharp |
| Forceps, thumb | 2 | One pair rat-toothed |
| Steel or stone for sharpening knives | 1 | |
| Hacksaw (with extra blades) | 1 | For removal of brain |
| Formaldehyde, 37% | 2 gal. | Dilute to a 10% solution with water for use |
| Alcohol | 5 gal. | Dilute to 70% with water for use |
| Plastic bags, 18 oz. whirlpack, 12" x 18" | 500 | |
| Centrifuge | 1 | For serum/plasma separation and microhematocrit determination |
| Syringes (35 ml, disposable) | 30 | |
| Needles (18 gauge x 3-1/2") | 6 | Clean and soak in 95% alcohol before reuse |
| Blood tubes: EDTA | 30 | |
| Heparin | 75 | |
| Plain | 150 | |
| Microhematocrit | 200 | |
| Screw cap vials (4 dram) | 1 gross | Serum/plasma storage |
| Balance | 1 | Organ weights |
| Tape measure, 3 m | 1 | |
| Microscope, 430 x, minimum | 1 | |
| Hemacytometer | 2 | |
| Captive bolt pistol | 1 | For sacrificing seals |

Appendix D.--Continued.

| Item | Quantity (minimum) | Remarks |
|---|---|---|
| Cap-chur dart pistol | 1 | Delivery of restraint drugs |
| Blood counting supplies (white cell Unipette) | 50 | |
| Microscope slides | 1 gross | |
| Differential blood cell stain | 1 pt | |
| Staining jar | 2 | |
| Culture vials with holding medium: Bacterial | 100 | |
| Viral | 100 | |
| Swabs, sterile | 200 | |
| Scapel blades (#21) | 100 | |
| Sample identification: | | |
| Labels | 500 | |
| Waterproof tags | 100 | |
| Pasteur pipettes, 6" | 100 | |
| Rubber bulbs for above | 6 | |
| Wood application sticks | 1 box | |

**U.S. DEPARTMENT OF COMMERCE**
**National Oceanic and Atmospheric Administration**
Western Administrative Support Center
7600 Sand Point Way N. E.
BIN C15700
Seattle, WA 98115

June 11, 1984

TO: F/SWC2 - Richard S. Shomura

FROM: RAS/WC3 - Robert J. Henderson Robert J Henderson

SUBJECT: Charter Vessel for Monk Seal "die-off" Response Plan

Your memo of May 21, 1984 requests that a mechanism be established to obtain vessel charter services on an emergency basis in the event of a monk seal "die-off."

During a telephone discussion of your needs between Bill Gilmartin and Brenda Lee Baker of my staff, it was determined that the smallest vessel that could be used is approximately 50 feet in lenth with a fuel capacity to travel from port of origin to Midway. Because the vessel will only be required to transport the scientific party we cannot use a fishing vessel but must use a vessel licensed to carry passengers for hire. There are four individuals/vessels in Hawaii that have expressed an interest in responding to our charter requirements but it is unknown if any of these are commercially licensed; three are licensed fishing vessels.

Your uncertain need to actually charter a vessel limits the alternatives available to us within the procurement regulations.

Basic agreements, basic ordering agreements, and indefinite delivery contracts are all mechanisms to be used when the exact needs of the government are unknown but are all predicated on orders actually being placed during the term of the agreement. Since you have not had an actual need for charter services during the past six years and have no anticipated need in the foreseeable future those mechanisms are not suitable for your requirements.

It appears at the present time the best strategy would be to identify prospective vessels that are commerically licensed for hire and would be interested in contracting with us. Emergency procedures could then be used to negotiate a contract should an actual need arise.

In the event a charter vessel is required, you may telefax your requirement giving the following pertinent information:

a. Description of size of vessel and estimated fuel capacity
b. Location and estimated duration of charter
c. Distance to be travelled from point of origin to destination
d. Number in the scientific party for accommodation purposes
e. Any special equipment needed
f. Funding citation and approval signatures; follow-up with hard copy requisition, SEC 970 and emergency justification by express mail

Under emergency provisions, the requirement would be immediately negotiated and a binding agreement consumated within seven days.

In light of the uncertainty of your requirements this is the best alternative at this time. We will endeavor to identify potential contractors and establish firm commitments suitable to your needs. If you need further assistance, please contact me or Brenda Lee Baker at FTS 392-6028.

Appendix F

**UNITED STATES DEPARTMENT OF COMMERCE**
**National Oceanic and Atmospheric Administration**
NATIONAL MARINE FISHERIES SERVICE
Southwest Fisheries Center
P.O. Box 271
La Jolla, California 92038

December 2, 1982

TO: F/SWC2 - William G. Gilmartin

THROUGH: F/SWC2 - Richard S. Shomura

FROM: F/SWC for Izadore Barrett

SUBJECT: Monk Seal Die-off Response

In response to your November 17 memo requesting help in identifying where the response money will come from, the SWFC can handle the lower end of the range (10K and thereabouts) from my reserve. For anything above this, I'm going to have to request funds from headquarters. I've alerted Dick Roe to this possibility, but have no firm commitment. Let's hope the monk seals stay reasonably healthy, so we never have to test the beyond-10K funding source.

cc: F/M4 - Richard B. Roe
F/SWC - Benjamin F. Remington

Appendix G

# AUTOPSY PROCEDURE FOR PINNIPEDS AND SMALL CETACEANS

By

Dr. Lynn A. Griner
Zoological Society of San Diego

June 1978

1. Examine the external surfaces of the body and describe any abnormalities, such as scars, active lesions, for example: "circular pale denuded area 1 cm in diameter with a central red foci." The anatomical location of the lesions should be given. Skin should be examined for external parasites and a sample of the parasite should be collected.

2. The animal to be autopsied should be placed on its back, and with a pointed knife make a midline incision through the skin on the ventral surface of the body, from the chin to the anus. The incision can best be made by pushing the knifepoint through the skin and then cutting the skin from the inside outward. If the cut is made from the outside, hair on the outside will take the edge off the knife very rapidly. Next, extend the incision through the blubber to the abdominal muscles; measure the thickness of the blubber at two or more points along the midline incision. Then with the knife reflect the skin and blubber laterally to expose the full ventral aspect of the animal. Blubber near the anus and genitalia should be incised and checked for the presence of small cysts which may contain yellowish colored parasites. This is especially true in cetacea.

   Then starting near the anterior edge of the pelvis, cut an oval shaped incision through the abdominal wall to the last rib on each side. Continue this incision anteriorly to the thoracic inlet by cutting through the cartilaginous part of the ribs near its junction with the bony portion of the rib. Then lift the posterior end of the abdominal wall anteriorly until the diaphragm is exposed. An incision should then be made through the diaphragm where it joins the abdominal muscles. The entire ventral wall of the animal can then be removed. Care should be taken not to cut through the sac surrounding the heart. Grossly examine the two cavities and make note of any abnormality observed, such as increased amount of fluid, peritonitis or pleuritis.

3. An incision should be cut through the soft tissues along the inner aspect of the lower jaw and cut the tongue loose from its anterior attachments. Then take hold of the tongue and cut through the hyoid bones and other soft tissues, so that the tongue, esophagus, and trachea are freed from the neck muscles. Continue cutting through this soft tissue until the above organs and the lung and heart are removed from the body cavity. The esophagus and aorta should be transected at the point where they pass through the diaphragm.

4. Examine the oral cavity and pharynx tonsillar area, paying close attention for the presence of mites or other parasites that might be present. The mites are small and usually white or grayish in color. If present, a few should be collected and preserved.

5. If the tonsils are enlarged, remove them and fix in Formalin.

6. The thyroid glands, which are located on the ventral lateral aspect of the larynx, near the origin of the trachea, should be examined and removed and fixed in Formalin.

7. The pericardial sac can then be opened...attention should be given to whether an excess of fluid is present; also note the color and consistency of any fluid that may be present.

   Examine the outer surface of the heart, check to see if there is any fat present along the line of juncture between the atria and ventricles. The heart of most pinnipeds is somewhat flattened and there is usually an indentation at the apex, where the right and left ventricles join. The wall of the right ventricle should be thinner and more relaxed than the left. Make an incision through both the right and left atria and ventricles. The right ventricle should be closely examined for the presence of parasites. The parasite most commonly found would be a very thin, elongated, threadlike roundworm. The worms may also be found up into the pulmonary artery and can extend all the way to the lungs. If worms are present, save a sample. The valves of the heart should be examined. Normally they are smooth, shiny, and white.

   If lesions are present they should be removed by cutting a slice of the affected tissue, approximately 1 cm in thickness. Be sure that some of the adjacent normal tissue is included. The aorta and other major arteries should then be opened and examined for abnormalities. Open and remove the esophagus and check for any abnormalities.

8. The respiratory system can be examined by incising the larynx and trachea, again looking for parasites. The surface of the lungs should also be examined for the presence of exudates, adhesions or any other lesion that are present. The color of the lungs should be observed; normally the pinniped lung collapses to a moderate degree when the thoracic cavity is opened. Look for small white nodules, which are frequently located under the pleura of the lung. If present, cut a piece of tissue approximately 1 × 2 × 2 cm in size from each lung, and fix. You can then use thumb forceps and try to tease open the white nodules and look for the presence of a very thin threadlike worm. The lung should be palpated for any firm areas that might be felt. If there are firm nodules, cut a small piece of tissue for microscopic examination. The bronchi should then be incised, with the incision extending throughout most of the lung.

9. An examination of the digestive system would require the removal of the liver, which should then be examined closely for abnormalities. The liver is usually reddish-brown in color and has a smooth surface.

The gall bladder should be opened and its contents noted. There are two things to look for, either in the gall bladder or in the bile ducts... they are, flukes and calculi, or stones.

Incision should be made into some of the larger bile ducts and again examined for flukes. The liver should then be sliced at approximately 1-cm intervals and gentle pressure should then be placed on the liver tissue to see if parasites can be extruded on to the cut surface. A 1 × 2 × 2 cm slice of the liver should be retained for microscopic study.

Cut the stomach loose from its attachment to the diaphragm and mesenteric connections, then transect the colon near the anus and cut all mesenteric attachments of the stomach and intestine and remove this group of organs from the abdominal cavity.

The pancreas, which is located near the juncture of the stomach and the small intestine, will appear as a pinkish-tan, somewhat lobulated tissue. This should be examined and a section saved.

With a knife or enterotome, open the stomach along its greater curvature. When incised, the contents of the stomach should be examined and it is recommended that some of the food material be saved for identification. The mucosal surface of the stomach is arranged in many folds which form rugae, somewhat resembling the surface of the brain. Roundworms are frequently found in the stomach of pinnipeds, so special attention should be made for parasites, and some of them should be saved for identification.

Examine the mucosal lining of the stomach carefully for ulcers. These may be small to large, and often will contain numerous parasites attached to the tissue. Examine the ulcers closely for hemorrhages, which may appear either as red hemorrhagic areas or they may be black due to the action of the stomach acid on blood. If possible, count the total number of ulcers, and the larger ones should be measured. Sections of the stomach through areas of ulceration should be saved for microscopic studies.

The intestine of pinnipeds and most sea mammals is very long, often over 100 ft. in length. Intestines can be opened longitudinally with the enterotome and it is much easier if the intestine is freed from its mesenteric attachment. The mucosal surface of the intestine should be examined as the incision progresses posteriorly. Samples of parasites should be collected. If ulcers are present in the intestine, a section of the tissue should be retained. Any other abnormalities, such as swelling, nodules, should also be retained. Often towards the more posterior part of the intestine can be seen small yellowish comma-shaped bodies; these are thorny-headed worms, and a section of the tissue with the attached worm should be retained. Several of the parasites should also be picked from the surface and fixed for identification.

10. The hematopoietic system includes the spleen and lymph nodes and bone marrow. The spleen and lymph nodes should be examined for enlargement, areas of hemorrhage, abscesses, and portions of abnormal tissue should be retained. Bone marrow will be mentioned under the musculoskeletal system.

11. The urogenital system includes the kidneys, ureters, urinary bladder, and sex organs. Immediately anterior to the kidneys you should find a pair of adrenal glands; they are usually round or oval in shape; however, in some animals they may be somewhat flattened, have the gross appearance of a lima bean. An incision through the gland will reveal a medullary zone which is tan in color, and an outer cortical zone that is reddish-brown.

    The kidneys are composed of numerous lobules, closely compressed together. Each lobule is like an individual kidney. An incision should be made through the kidney and along the cut surface look for the presence of any calculi or stones which are located in the calyces of the lobules. Remove the kidney capsule and look for pale embedded areas. Pieces of the kidney, with or without lesions, should also be retained for microscopic study.

    Incise the urinary bladder and note the color and amount of urine present, and again look for the presence of stones.

    The female genitalia, including vagina, cervix, and uterus, should be opened and examined, and the ovaries should be collected and placed in Formalin. Likewise in the males the testicles should be removed and incised; a sample of the organ should be retained.

12. Musculoskeletal system: Pinniped cetacean muscles are dark red in color, and they have a stringy gross appearance. Examine the muscles and especially the fascial planes that divide one muscle from another. Along the fascial planes of some of the abdominal muscles can occasionally be found filarial roundworms. If present, they should be collected. If the animal is pale or anemic in appearance, one of the long bones, such as the femur, should be removed and incised, and a portion of the bone marrow should be collected and placed in Formalin.

13. Nervous system: The brain and spinal cord are difficult to remove. In order to obtain the brain you should remove the head by cutting through the joint that attaches the head to the vertebrae. Then reflect the skin from the back and top of the skull forward. By means of a hacksaw cut a circle through the top of the skull so that it will connect with the large foramen at the base of the brain. This is the foramen through which the spinal cord leaves the brain and enters the vertebral column. The anterior part of the incision through the skull should be back of the bony orbit. Remove the top of the skull and remove the brain. This can best be done by means of scissors. The meninges that enclose the brain are very tough and it is necessary to cut them and not try to pull the brain free. If there is reason to believe a nervous disturbance was present, the entire brain should be fixed in Formalin; otherwise, cut two or three slices from the cerebrum and also frm the cerebellum. Also save a portion of the spinal cord where it joins the brain.

On the ventral surface of the brain you should be able to find the pituitary gland. This should also be retained along with the adrenals that were mentioned earlier, as they represent some of the endocrine organs.

14. All of the saved tissue should be placed in Formalin, and it is advised that the Formalin be replaced at 24 or 48 hours. A week or 10 days later the fixed tissues can be removed from the Formalin and placed in flip-top plastic bags, along with a piece of gauze and 3 or 4 tablespoons of Formalin. The bags should then be sealed and they can be stored until they can be sent to the laboratory. The tissues should not be placed in direct sunlight. The tissues from each necropsy should be identified with the proper label or tag. On the completion of the autopsy, a complete description should be written. This report should describe any lesions observed. The tissues, along with a copy of the autopsy report, should then be submitted to a pathology lab for histopathologic study.

# Appendix H

MASS MORTALITY SPECIMEN COLLECTION GUIDELINES

| Specimens | Preparation/Storage | Tests | Remarks |
|---|---|---|---|
| 1. Pathology Tissue Set (to include representative normal and abnormal samples collected as per guidelines in "Autopsy Procedure for Pinnipeds and Small Cetaceans" - Griner) | 10% buffered Formalin (at least 10 × tissue volume) | Microscopic histopathologic examination | Collect tissue as soon as possible after death, but not after 24 h. |
| 2. Toxicology Tissue Set (approximately 100 g of these tissues)<br>Liver<br>Brain<br>Kidney<br>Muscle<br>Blubber | Wrap in aluminimum foil, seal in plastic bags, and freeze | Ciguatera; metals; chlorinated hydrocarbons; others as indicated | |
| 3. Parasites | Place all parasites recovered into alcohol separated by collection site | Identification; quantification | |
| 4. Blood, 75 ml | 5 ml with EDTA<br>20 ml with heparin - separate plasma and freeze<br>50 ml without anticoagulant - separate serum and freeze | Blood counts; electrolytes; reproductive hormones; enzymes; other chemistry as indicated; antibody titers | Only from live animal or immediately after death |
| 5. Skull | Flense and dry or freeze | Measurements; age | |
| 6. Stomach contents | Freeze or place in 5 volumes of alcohol | Identification of contents; ciguatoxin analysis | |